

Cyberlindnera jadinii yeast as a protein source for broiler chickens: effects on growth performance and digestive function from hatching to 30 days of age

Ana Cruz,^{*,†} Hallgeir Sterten,^{*} Franciska S. Steinhoff,^{*} Liv T. Mydland,[†] and Margareth Øverland^{†,1}

^{*}Felleskjøpet Fôrutvikling A.S., Trondheim NO-7018, Norway; and [†]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO 1432 Ås, Norway

ABSTRACT Europe is heavily dependent on imported feed protein sources such as soybean meal (SBM); thus, investigating local sustainable alternatives is crucial to increase self-sufficiency. This study evaluated the effects of the inactivated yeast *Cyberlindnera jadinii* grown on local lignocellulosic sugars on the growth performance and digestive function of Ross 308 broiler chickens. A total of 1,000 male chicks were allocated to 20 pens. There were 5 replicate pens with 50 birds each, from 1 to 30 D after hatch. The birds were offered one conventional wheat–oat–SBM–based control diet and 3 diets with increasing levels of *C. jadinii* replacing 10, 20, and 30% of dietary crude protein (CP), whereas SBM levels were gradually decreased. The feed intake and weight gain of the birds decreased linearly, and feed conversion ratio increased linearly ($P < 0.01$) with increasing dietary levels of *C. jadinii*. Nevertheless, growth performance and feed intake were similar between the birds fed with control

diets and diets containing 10% CP from *C. jadinii* in the starter and grower periods. The apparent ileal digestibility (AID) of dry matter, crude fat, organic matter, and carbohydrates was higher in control diets than in diets with 30% *C. jadinii* CP ($P < 0.05$) and decreased ($P < 0.01$) with incremental levels of dietary *C. jadinii*. Regardless, the AID of CP, starch, ash, and phosphorus was unaffected. Ileal villus height on day 10 was maintained in birds fed with diets containing 30% *C. jadinii* CP compared with the birds fed with control diets but was lower for birds fed with diets containing 10 and 20% *C. jadinii* protein ($P < 0.05$). To conclude, up to 10% *C. jadinii* CP can replace SBM CP in broiler chicken diets, maintaining growth performance and digestive function, whereas higher levels of *C. jadinii* may decrease bird performance. Altogether, this suggests the potential of *C. jadinii* as a local-based protein source in broiler chicken diets, contributing to a more sustainable feed.

Key words: alternative protein source, intestinal morphometry, digestibility, Ross 308, production parameter

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INTRODUCTION

The global poultry production has rapidly increased over the last decades, and the demand for poultry meat is expected to increase in the next years. On average, a European person currently consumes around 25 kg of poultry meat annually (OECD and FAO, 2018). Europe is heavily dependent on imported feed protein sources such as soybean meal (SBM), but to increase self-sufficiency, it is important to develop local

sustainable high-quality protein sources. Recently, there is an increased interest in alternative protein feedstuffs, such as the inactivated yeast *Cyberlindnera jadinii* (previously classified as *Candida utilis*) produced on sugars from lignocellulosic biomass such as the Norwegian spruce tree (*Picea abies*; Øverland and Skrede, 2017). *C. jadinii* is a potentially sustainable alternative protein source with a low carbon footprint compared with soy protein concentrate (Couture et al., 2019). Yeasts have mostly been used as additives, in small amounts in animal feed, for their functional properties, but limited information exists on the use of yeast as a protein source. Different yeast strains such as *C. jadinii*, *Saccharomyces cerevisiae*, and *Kluyveromyces marxianus* have recently been evaluated as replacement for high-quality fish meal in diets for Atlantic salmon (*Salmo salar*), with highest growth performance for *C. jadinii*-containing diets, owing to a high crude protein (CP) content of 56%

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¹Corresponding author: margareth.overland@nmbu.no

and a high CP digestibility (Øverland et al., 2013). The use of *C. jadinii* as a feed additive has been extensively studied (Shurson, 2018). The yeast *C. jadinii*, representing 20 to 40% of the CP in monogastric animal diets, has shown to increase feed intake and growth rate in broiler chickens (Rodríguez et al., 2013) and shown to maintain growth performance and improve digestive function in weanling piglets (Cruz et al., 2019). In addition, 40% of CP from *C. jadinii* in diets has shown to maintain growth performance in Atlantic salmon (*S. salar*) (Øverland et al., 2013) compared with a fish meal-based control. *C. jadinii* also contains a wide range of bioactive components such as β -glucans, mannoooligosaccharides, and nucleic acids (Maul et al., 1970; Nguyen et al., 1998; Kogan and Kocher, 2007) shown to improve digestive function and health of farm animals, especially during critical life stages (Gopalakannan and Arul, 2009; Grammes et al., 2013; Cruz et al., 2019; Hansen et al., 2019). Altogether, this suggests that *C. jadinii* can potentially serve as a local-based and sustainable protein-rich ingredient in animal feed. The present

study was therefore performed to evaluate the effects of *C. jadinii*, grown on lignocellulosic sugars, on production parameters and intestinal morphometry of broiler chickens, when gradually replacing SBM in broiler chicken diets.

MATERIALS AND METHODS

Diets and Experimental Design

The diets were formulated to meet or exceed the requirements for protein, essential amino acids (AAs), and all other nutrients. All diets were isoenergetic; isonitrogenous; equal in digestible lysine, methionine and cysteine, threonine, tryptophan, and arginine per energy unit; and equal in the concentration of Na, Ca, and P. In addition, all diets were free from coccidiostats (Tables 1 and 2). The dietary treatments consisted of 4 diets for the starter period (day 0–9) and 4 diets for the grower period (day 10–30), for both periods including a control diet based on wheat, oats, and SBM (control) and 3 diets

Table 1. Ingredient composition and calculated content (g/kg, unless otherwise stated) of experimental diets fed to broiler chickens from day 1 to 30 after hatching.

Ingredients %, as is	Starter diets ¹				Grower diets ¹			
	Control	CJ10	CJ20	CJ30	Control	CJ10	CJ20	CJ30
Wheat	52.8	53.6	54.5	55.3	62.5	63.1	63.7	64.3
Oats	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
<i>Cyberlindnera jadinii</i> ²	0.0	4.9	9.8	14.7	0.0	4.2	8.3	12.5
Soybean meal	15.3	10.2	5.1	0.0	12.6	8.4	4.2	0.0
Fish meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Rapeseed meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Potato protein concentrate ³	5.0	5.0	5.0	5.0	3.0	3.0	3.0	3.0
Maize gluten meal	5.0	5.0	5.0	5.0	2.0	2.0	2.0	2.0
Soy oil	4.3	3.6	3.0	2.3	2.5	2.0	1.5	1.0
Vitamin and trace mineral mix ⁴	0.64	0.63	0.63	0.63	0.62	0.63	0.63	0.63
Limestone meal	0.95	0.95	0.92	0.91	0.8	0.79	0.78	0.77
Monocalcium phosphate	0.67	0.78	0.88	0.99	0.46	0.55	0.63	0.72
Sodium bicarbonate	0.41	0.33	0.24	0.16	0.4	0.31	0.23	0.14
Sodium chloride	0.01	0.01	0.00	0.00	0.06	0.06	0.06	0.06
L-Lysine	0.38	0.33	0.29	0.25	0.38	0.34	0.3	0.26
L-Methionine	0.26	0.28	0.29	0.30	0.27	0.28	0.29	0.3
L-Arginine	0.15	0.20	0.24	0.28	0.16	0.19	0.23	0.26
L-Threonine	0.12	0.10	0.08	0.07	0.15	0.13	0.11	0.09
L-Tryptophan	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.01
Enzymes	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Titanium dioxide (TiO ₂)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Calculated content								
Metabolizable energy ⁵	2,892	2,892	2,892	2,892	2,820	2,820	2,820	2,820
Crude protein	23.5	23.5	23.5	23.5	20.0	20.0	20.0	20.0
Crude fat	59.4	57.4	55.5	53.5	42.5	39.2	35.8	32.5
CP from <i>C. jadinii</i>	0.0	10.0	20.0	30.0	0.0	10.0	20.0	30.0
Amino acids (%)								
Lysine	1.41	1.42	1.43	1.44	1.22	1.23	1.23	1.24
Methionine	0.68	0.69	0.71	0.72	0.60	0.61	0.62	0.63
Cysteine	0.37	0.36	0.34	0.32	0.37	0.32	0.30	0.29
Threonine	0.95	0.95	0.95	0.96	0.83	0.83	0.83	0.83
Tryptophan	0.27	0.27	0.27	0.27	0.24	0.24	0.24	0.24
Arginine	1.45	1.46	1.46	1.47	1.27	1.27	1.27	1.27

¹Control diet (control); diets with 10, 20, and 30% crude protein (CP) from *C. jadinii* (CJ10, CJ20, and CJ30, respectively).

²Dried inactivated *C. jadinii* (%): DM = 97.0, CP = 47.0, crude fat = 1.6, ash = 7.8, gross energy = 4,756 kcal/kg; essential amino acid content in grams per 16 g N: Arg = 24.4, His = 8.5, Ile = 21.6, Leu = 31.6, Lys = 30.6, Met = 5.2, Phe = 18.4, Thr = 25.6, Val = 25.9, Trp = 6.2.

³Cargil, Denmark.

⁴Vitamin–trace mineral premix, provided per 1 kg of diet: vitamin A = 9,600 IU; dl- α -tocopheryl acetate = 100 mg; cholecalciferol = 5,000 IU; menadione = 6 mg; thiamin = 3.9 mg; riboflavin = 7.4 mg; pantothenic acid = 59 mg; niacin = 20 mg; pyridoxine = 12 mg; biotin = 0.4 mg; cyanocobalamin = 20 μ g; betaine = 1.1 g; selenium = 0.29 mg; Fe (FeSO₄) = 67 mg; Mn (MnO) = 127 mg; Zn (ZnO) = 60 mg; Cu (CuSO₄) = 11 mg; I (Ca [IO₃]) = 1.28 mg.

⁵Apparent metabolizable energy, values in kilocalorie per kilogram, calculated based on Centraal Veevoederbureau (2005).

Table 2. Analyzed chemical composition of experimental diets.

Composition, %	Starter diets ¹				Grower diets ¹			
	Control	CJ10	CJ20	CJ30	Control	CJ10	CJ20	CJ30
Dry matter	91.10	91.30	91.90	92.30	89.30	90.00	90.20	90.80
Ash	5.30	4.90	5.00	5.10	4.20	4.40	4.50	4.40
Crude protein ²	25.80	25.80	26.20	25.90	23.20	23.30	23.30	23.10
Starch	37.10	38.90	40.10	40.30	40.30	42.30	44.00	44.20
Crude fat	5.50	5.50	4.80	4.50	4.80	4.30	4.10	3.60
Crude fiber	3.50	3.40	3.30	3.20	3.30	3.40	3.00	3.20
Phosphorus	0.60	0.66	0.72	0.76	0.56	0.52	0.63	0.68
Potassium	0.83	0.85	0.86	0.89	0.76	0.78	0.79	0.82
Calcium	0.99	0.83	0.79	0.81	0.62	0.61	0.68	0.64
Sodium	0.20	0.17	0.14	0.12	0.16	0.16	0.15	0.14
Essential amino acids								
Arginine	1.40	1.33	1.31	1.29	1.23	1.20	1.17	1.11
Histidine	0.54	0.53	0.50	0.48	0.48	0.46	0.43	0.40
Isoleucine	0.92	0.92	0.89	0.88	0.74	0.72	0.69	0.68
Leucine	1.85	1.86	1.88	1.81	1.55	1.53	1.51	1.40
Lysine	1.37	1.33	1.28	1.23	1.20	1.17	1.12	1.07
Methionine	0.60	0.60	0.62	0.66	0.59	0.59	0.56	0.57
Phenylalanine	1.13	1.13	1.08	1.06	0.93	0.90	0.86	0.82
Threonine	0.90	0.90	0.93	0.91	0.82	0.79	0.79	0.74
Valine	0.84	0.85	0.85	0.90	0.75	0.74	0.75	0.74
Tryptophan	0.27	0.25	0.26	0.27	0.25	0.22	0.24	0.24
Nonessential amino acids								
Alanine	0.96	0.96	1.00	1.02	0.77	0.78	0.81	0.80
Asparagine	1.98	1.90	1.85	1.68	1.60	1.51	1.46	1.35
Glycine	0.90	0.83	0.82	0.81	0.74	0.72	0.71	0.69
Glutamate	4.90	4.87	4.83	4.73	4.60	4.52	4.40	4.21
Cysteine	0.33	0.34	0.31	0.29	0.31	0.28	0.27	0.26
Tyrosine	0.70	0.68	0.69	0.70	0.42	0.42	0.42	0.44
Proline	1.51	1.36	1.47	1.47	1.37	1.30	1.35	1.32
Serine	1.00	1.00	1.02	0.97	0.89	0.85	0.85	0.80
Total amino acids	21.85	21.39	21.33	20.91	18.97	18.46	18.15	17.38

¹Control diet based on soybean meal, wheat, and oats (control); diets with 10, 20 and 30% crude protein from *C. jadinii* (CJ10, CJ20, and CJ30, respectively).

²Pregl-Dumas (N × 6.25).

where 10, 20, and 30% of the CP originating from SBM was replaced by CP from *C. jadinii* (CJ10, CJ20, and CJ30, respectively). The experimental diets were produced at the Center for Feed Technology (FôrTek), As, Norway. The starter and grower diets were pelleted at a diameter of 2.5 and 3.5 mm, respectively, at the minimum pelleting temperature of 82°C for microbial control. Each diet was added with 0.06% titanium dioxide (TiO₂) as a digestibility marker. Representative samples of main raw materials and finished feed were collected in duplicate from each diet. Pellet hardness was evaluated using the pellet durability index (Holmen NHP200, UK) in 3 replicates per diet, at the Feed Quality Laboratory, Norwegian University of Life Sciences, As, Norway.

One thousand male Ross 308 broiler chickens were distributed in 20 pens of dimensions of 5.3 m², each containing 50 birds, constituting a total of 5 replicates per diet. The mean initial BW was 42.0 g ± 0.75 standard deviation in all treatments.

Bird Management

All birds were handled and cared for as per local welfare laws and regulations, the Animal Welfare Act of December 28, 2009, and the local legislation derived from the directive 2010/63 EU of the European Parliament and Council of September 22, 2010, on the protection of animals used for scientific purposes.

All birds had *ad libitum* access to feed and water. The bedding (or litter) used in the pens was composed of wood shavings. The starter feed was provided from day 0 until day 9, and the grower feed was provided from day 10 to slaughter (day 30). Accumulated feed intake was recorded for each pen weekly, and live BW were registered for each pen weekly and at slaughter. Room temperature was kept on average at 32°C, 28°C, 25°C, and 23°C from days 1 to 7, 8 to 14, 15 to 21, and 22 to 28, respectively. During the first 24 h, the birds were kept in 23-h light and 1-h darkness cycles, and during the remaining period (day 2–30), they were kept in 16-h light and 8-h darkness cycles. The relative humidity (%) was on average 26.3, 35.9, 39.0, and 41.6 for days 1 to 7, 8 to 14, 15 to 21, and 22 to 28, respectively. Litter quality was scored weekly for each pen on days 4, 11, 18, 25, and 28 on a 0 to 5 scale based on the visual and tactile estimation of water content, in which 1 was considered completely dry and 5 was considered completely wet. Mortality was calculated and recorded daily for all pens, and water intake was measured daily for 10 of the pens. At slaughter (day 30), all birds were inspected for general health (footpad lesions, systemic diseases, ascites), and the eviscerated carcass weights were registered individually. Footpads were scored as 0, 1, or 2 based on the presence and severity of lesions (0, normal, no lesions; 1, mild or superficial damage, darkened areas; and 2, severe ulceration with possible swelling or bleeding).

Yeast Microbial Ingredient

C. jadinii (LYCC 7549; Lallemand Yeast Culture Collection) was produced by Lallemand Inc., Saluta-guse, Estonia. Second-generation sugars were obtained from lignocellulosic biomass of Norway spruce trees (*P. abies*) by using the Borregaard Advanced Lignin Process at Borregaard AS, Sarpsborg, Norway (Patent “Lignocellulosic biomass conversion by sulfite pretreat-ment;” EP2376642B1 EP Grant). The C5 and C6 sugars were used in the growth media for the yeast, as described by Øverland and Skrede (2017) and Sharma et al. (2018). The chemical composition of *C. jadinii* is presented as a footnote in Table 1.

Sample Collection

Ten birds per diet were euthanized by cervical disloca-tion on day 10 and day 28 for sample collection. The abdominal cavity was sectioned caudal to the sternum, and the ileum, ceca, and Meckel’s diverticulum were located and exposed. On day 10, tissue from the duo-denum, jejunum, and ileum was collected from 10 birds per dietary treatment for intestinal morphometry mea-surements. Four- to five-cm pieces of the distal duo-denum, jejunum, and ileum were collected, rinsed, and placed in buffered formalin solution (10%). On day 28, individual ileal content samples were collected from the intestinal segment between Meckel’s diverticulum and the ileocecal junction for the measurement of apparent ileal digestibility (AID) of nutrients of 10 birds per diet. The digesta samples were placed into locked plastic containers, preserved on ice for transportation, and stored at -20°C until freeze-drying.

Chemical Analysis

The chemical composition of the experimental diets and the raw materials are presented in Table 2. Ileal digesta samples from 2 birds per pen were pooled before analysis ($n = 5$ pens per diet) owing to the small quantity of each individual sample to ensure enough material for chemical analysis. The pooled samples were ground through a 1-mm and 0.5-mm screen, homogenized, and analyzed in triplicate for dry matter, ash, starch, nitro-gen, crude fat, and TiO_2 . Diets were freeze-dried, ground at particle sizes of 1 mm and 0.5 mm (0.5 mm for nitrogen analysis), homogenized, and analyzed in duplicate for dry matter, ash, CP, starch, and crude fat and in triplicate for phosphorus and TiO_2 . Dry matter, ash, and AA were determined as per the methods described in the European Commission Regulation (EC) No 152/2009 (European Commission, 2009). Nitrogen was analyzed using the Pregl-Dumas method: freeze-dried ileal content and diets were submitted to combustion at $1,150^{\circ}\text{C}$ (Elemental Analysesysteme GmbH, Hanau, Germany), and N_2 mole-cules were separated and measured by thermal conductiv-ity detection. Crude fat was determined by accelerated solvent extraction (ASE350, Dionex Corporation, Sunny-va-le, CA): feed samples were extracted with 70%

petroleum ether and 30% acetone at 125°C ; ileal samples were extracted with 80% petroleum and 20% acetone at 125°C . Starch was hydrolyzed with α -amylase and amylo-glucosidase enzymes to glucose, and glucose concentra-tion was determined using a spectrophotometer (MaxMat PL II Multianalyzer, France) as described by McCleary et al. (1994). Phosphorus was analyzed using the spectrophotometric method, as per the Commission Regulation (EC) No 152/2009 and ISO 6491. Crude fiber, calcium, sodium, and potassium were analyzed at Euro-fins Food & Feed Testing AS, Moss, Norway. Crude fiber was analyzed as per the method (Fibertec) ISO 5498. Cal-cium, sodium, and potassium were analyzed as per the method ISO 17294-2. TiO_2 concentrations in ileal samples and diets were determined using the method described by Short et al. (1996).

Gut Morphometric Indices

Formalin-fixed samples were dehydrated in graded levels of alcohol, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Four histological cuts per animal were used for measurements. The ileal villi heights (VH) and crypt depths (CD) were measured using ViewPoint version 0.8.2.7 software (PreciPoint GmbH, Freising, Germany) on images captured from an Olympus BX43 light microscope (Olympus Norge AS, Asker, Nor-way) mounted with the IDS UI3260CP-C-HQ 2.3 MP camera (IDS Imaging Development Systems GmbH, Obersulm, Germany) using the whole-slide scanning software, MicroVisioneer (MicroVisioneer, Freising, Ger-many). The 5 longest and well-oriented villi in proximity to well-oriented crypts were selected from each individual, and micrographs were captured using a $20\times$ objective magnification. The 5 crypts adjacent to those villi, in the same micrographs, were selected for measurements of CD. The VH were measured by drawing a line through the center of the villus extending from the tip of the mucosal epithelium to the villus–crypt junction. The CD were measured from the villus–crypt junction to the tunica muscularis mucosae. The VH-to-CD ratios were calcu-lated using the mean VH and mean CD of 5 observations per bird where possible, with a minimum of 3 observations per bird.

Calculations and Statistical Analysis

Feed conversion ratio (FCR) was calculated as fol-lows: $\text{FCR} = \text{total feed intake (g)}/\text{total live weight gain (g)}$. Live weights on days 10 and 30 were estimated based on a growth curve $y = ax^3 + bx^2 + cx + d$, estab-lished from average live weights for each pen on days 0, 7, 14, 21, and 28. The AID of dry matter, starch, CP, and crude fat was calculated as follows: $\text{AID (\%)} = (1 - ((T_{\text{absF}})/T_{\text{absI}}) \times (N_{\text{il}}/N_{\text{f}})) \times 100$, where T_{absF} is the TiO_2 absorbance at 410 nm in the feed sample on a dry matter basis; T_{absI} is the absorbance of TiO_2 at 410 nm in the ileal sample on a dry matter basis; N_{il} is the concentration of a nutrient in the ileal sample on a dry matter basis; and N_{f} is the concentration of the

nutrient in the feed sample on a dry matter basis. The relationship between TiO_2 absorbance at 410 nm and TiO_2 concentration in a sample was described by Short et al. (1996). The carbohydrate fraction (CHO), which included lignin, was calculated as follows: $\text{CHO} = \text{dry matter} - \text{ash} - \text{CP} - \text{crude fat}$, where dry matter, ash, CP, and crude fat values resulted from the chemical analysis of feed and ileal content. Statistical analyses of growth performance were performed using the general linear model multivariate procedure in SPSS Statistics software, version 25.0 (IBM Corp., Armonk, New York, 2017). Tukey's honestly significant difference was used for multiple comparisons between the dietary treatments when statistical differences ($P < 0.05$) were observed. The statistical unit was the pen. Values for ADG, ADFI, and FCR were analyzed as per the model $Y_i = \mu + \alpha_i + \varepsilon_{ij}$, where Y_i is the dependent variable (pen), μ represents the overall sample mean, α_i is the effect of the dietary treatment ($i = 1, 2, 3, 4$), and ε_{ij} is the residual error. Linear, quadratic, and cubic polynomial contrasts were used to determine the effects of dietary yeast protein on growth performance, digestibility, and intestinal morphometry. The mortality rate (%) was calculated as follows: $\text{MR} = [(n_{t1} - n_{t2})/n_{t1}] \times 100$, where n_{t1} is the number of live birds at the time point t1 and n_{t2} is the number of live birds at time point t2. Statistical analysis of mortality rates was performed using the univariate general linear model procedure in SPSS. Values are presented as mean \pm standard deviation. Means for litter quality scores, room temperature, and relative humidity were calculated in Microsoft Excel 2016 using the command "average." Differences between the diet groups were investigated using the general linear model procedure in SPSS Statistics, considering a significance level of $P < 0.05$. Mean carcass weights were analyzed by the procedure "compare means" by one-way analysis of variance in SPSS.

RESULTS

Diets

The values for the calculated composition of the diets were based on the analyzed chemical composition. These are shown in Tables 1 and 2, respectively. In general, the postpelleting temperatures of the pellets gradually increased in the production of starter and grower diets with the addition of *C. jadinii*. The postpelleting temperatures were 100.3°C, 103.7°C, 104.9°C, and 110.3°C for the control, CJ10, CJ20, and CJ30 starter diets and 92.0°C, 95.0°C, 95.0°C and 105.0°C for the control, CJ10, CJ20, and CJ30 grower diets, respectively. In accordance with the pellet temperature, the pellet durability index increased with the addition of *C. jadinii* to the diets. The pellet durability index was 92.0, 93.3, 94.6, and 94.8 for the control, CJ10, CJ20, and CJ30 starter diets, respectively, and 87.5, 93.0, 93.4, and 95.7 for the control, CJ10, CJ20, and CJ30 grower diets, respectively. The pellet durability index values were found to be in the range of high pellet quality standards.

General Health

In general, the birds were healthy throughout the experiment, and the mortality rate in the control group was within the expected range of values for male broiler chickens in Norwegian poultry production. There was an increase in mortality with increasing levels of *C. jadinii* in the diets, although statistically, the mortality rate was not affected by dietary treatment ($P = 0.168$, Table 3). The overall mortality rate (day 0–30) was 4.0%. General health findings from the slaughter inspections are summarized in Table 4. During the routine veterinarian inspection of the slaughtered birds (day 30), some of the carcasses were rejected owing to observed clinical signs of disease, but no statistical relationships between these findings and the dietary treatments were found. There were no statistical differences in footpad lesions and in the litter score among the dietary treatments.

Growth Performance

The results for growth performance, water intake, carcass weights, and mortality rate are shown in Table 3. Final live BW was lower for the birds fed with the CJ20 and CJ30 diets than for the birds fed with the control and CJ10 diets ($P < 0.001$), and it linearly decreased with increasing levels of *C. jadinii* ($P < 0.001$). The BW gain decreased linearly with increasing levels of dietary *C. jadinii* during the starter period ($P < 0.01$), grower, and overall periods ($P < 0.001$). Birds fed with the CJ30 diet had lower BW gain than those fed with the control diet during the starter period ($P < 0.05$). Birds fed with the CJ20 and CJ30 diets had lower BW gain than those fed with the control and CJ10 diets during the grower and overall periods ($P < 0.001$). Feed intake did not differ among the dietary treatments during the starter period but was lower for the birds fed with the CJ20 and CJ30 diets than those fed with the CJ10 and control diets during the grower period ($P < 0.001$). During the overall period, feed intake was lower for birds fed with the CJ30 diet than for those fed with the control diet ($P < 0.01$). Feed intake decreased linearly with increasing levels of *C. jadinii* in the diets during the grower and overall period ($P < 0.001$), but not during the starter period. The FCR increased linearly with increasing levels of *C. jadinii* in the diets during the starter ($P < 0.001$), grower ($P < 0.05$), and overall ($P < 0.01$) periods. Birds fed with the CJ20 and CJ30 diets had higher FCR than those fed with the control diet during the starter period ($P < 0.01$). During the overall period, FCR was higher for birds fed with the CJ30 diet than for those fed with the control diet ($P < 0.05$). The FCR of birds fed with the CJ10 diet was similar to that of birds fed with the control diet during the starter period and was similar among the dietary treatments during the grower period. Average daily water intake did not differ among dietary treatments. The carcass weights of birds fed with the CJ10 diet were similar to those fed with the control

Table 3. Effects of the increasing dietary level of *Cyberlindnera jadinii* yeast protein on the growth performance of broiler chickens during the starter (day 0–10), grower (day 11–30), and overall (day 0–30) periods¹.

Item, g/bird	Diet ²				SEM ³	P-value	Linear
	Control	CJ10	CJ20	CJ30			
Start BW	42.1	42.2	41.6	41.8	0.38	0.647	0.378
Final live BW	1,987 ^a	1,935 ^a	1,824 ^b	1,699 ^b	36.11	<0.001	<0.001
BW gain							
Starter period	246 ^a	239 ^{a,b}	226 ^{a,b}	188 ^b	13.67	0.036	0.007
Grower period	1,699 ^a	1,653 ^a	1,555 ^b	1,469 ^b	24.89	<0.001	<0.001
Overall	1,944 ^a	1,892 ^a	1,782 ^b	1,657 ^b	36.14	<0.001	<0.001
Feed intake							
Starter period	275	288	296	254	12.31	0.124	0.334
Grower period	2,310 ^a	2,257 ^a	2,150 ^b	2,075 ^b	32.61	<0.001	<0.001
Overall	2,586 ^a	2,546 ^{a,b}	2,446 ^{a,b}	2,329 ^b	38.94	0.001	<0.001
FCR ⁴							
Starter period	1.12 ^b	1.21 ^{a,b}	1.32 ^a	1.36 ^a	0.04	0.004	<0.001
Grower period	1.36	1.37	1.38	1.41	0.02	0.183	0.038
Overall	1.33 ^b	1.35 ^{a,b}	1.37 ^{a,b}	1.41 ^a	0.02	0.027	0.004
Water intake ⁵ (mL/bird, grower)	3,742	3,887	3,577	3,402	145.82	0.248	0.111
Water:feed intake (grower)	1.57	1.60	1.54	1.57	0.04	0.791	0.820
Carcass weight ⁶	1,225 ^a	1,188 ^a	1,115 ^b	1,030 ^c	10.57	<0.001	<0.001
Mortality rate (%)	2.0	3.6	4.4	5.6	1.79	0.561	0.168

^{a-c}Values in the same row with different superscripts differ ($P < 0.05$).

¹Values are presented as least-square means, ($n = 20$).

²Control diet based on soybean meal, wheat, and oats (control); diets with 10, 20 and 30% crude protein from *C. jadinii* (CJ10, CJ20, and CJ30, respectively).

³SEM: pooled standard error of the means.

⁴FCR: feed conversion ratio (gram feed per gram gain).

⁵Water consumption calculated from days 8 to 28 (mL/bird); $n = 10$.

⁶Defeathered, eviscerated.

diet ($P < 0.001$) but were lower for birds fed with the CJ20 and CJ30 diets. Carcass weight decreased linearly with increasing amounts of *C. jadinii* in the diet ($P < 0.001$). The mortality rate numerically increased with increasing levels of *C. jadinii* in the diets, but this did not reach statistical significance.

Apparent Ileal Digestibility of Nutrients

The AID of nutrients is shown in Table 5. The AID of dry matter and crude fat was lower in birds fed with the

CJ30 diet than in those fed with the control diet ($P < 0.05$) and decreased linearly with increasing levels of *C. jadinii* in the diet ($P < 0.01$). In addition, the AID of the carbohydrate fraction and organic matter was lower for the birds fed with the CJ30 diet than for the birds fed with the control diet ($P < 0.01$) and decreased linearly with increasing levels of *C. jadinii* in the diets ($P < 0.01$). The AID of CP, starch, ash, and phosphorus did not differ among the dietary treatments.

Gut Morphometric Indices

The results of the intestinal morphometry evaluation are shown in Table 6. Ileal VH on day 10 were similar in birds fed with the CJ30 diet and in those fed with the control diet but lower for the birds fed with the CJ10 and CJ20 diets ($P < 0.05$), presenting a quadratic trend ($P < 0.01$). Duodenal CD tended to decrease with increasing levels of *C. jadinii* in the diets ($P < 0.05$). Including *C. jadinii* in the diets led to a quadratic effect on the ileal absorption area ($P < 0.05$), wherein birds fed with the control and CJ30 diets had the highest ileal absorption area.

DISCUSSION

The present study suggests that *C. jadinii* is a potential alternative protein source that can replace up to 10% of CP from SBM in diets for broiler chickens, resulting in similar growth performance and nutrient digestibility compared with birds fed with control diets. The nutritional composition of yeast, including protein content, may vary depending on the strain, growth media, and

Table 4. Carcasses (%) rejected during the slaughter procedure inspection, footpad lesions, and litter quality scores of broiler chickens fed with a control diet and diets with increasing dietary levels of *Cyberlindnera jadinii* yeast.

Item	Diet ¹			
	Control	CJ10	CJ20	CJ30
Total inspected (n)	225	221	218	215
Total rejected (%) ²	2.2	5.4	4.1	6.5
General disease	0.4	2.7	1.8	2.8
Ascites	0.4	1.4	0.9	1.4
Peritonitis	0.0	0.9	0.5	1.4
Footpad lesions (%) ³	0.0	0.0	0.0	2.0 ³
Litter quality score ⁴				
Day 11	1.0	1.0	1.0	1.0
Day 18	1.7	1.8	1.8	1.8
Day 25	2.1	2.3	2.3	2.3
Day 28	2.5	2.6	2.6	2.6

¹Control diet based on soybean meal, wheat, and oats (control); diets with 10, 20 and 30% crude protein from *C. jadinii* (CJ10, CJ20, and CJ30, respectively).

²Calculated as the number of cases per number of inspected birds.

³Broiler chickens classified with mild footpad lesions.

⁴Categorized on a scale from 1 to 5 based on visual and tactile humidity and consistency (1 = completely dry, 5 = very wet).

Table 5. Effects of increasing dietary levels of *Cyberlindnera jadinii* on the apparent ileal digestibility of nutrients in broiler chickens¹.

AID, %	Diet ²				SEM ³	P-value	Linear
	Control	CJ10	CJ20	CJ30			
Dry matter	75.4 ^a	73.0 ^{a,b}	71.3 ^{a,b}	69.3 ^b	1.09	0.008	0.001
Crude protein ⁴	81.0	79.4	78.1	76.5	1.56	0.252	0.050
Starch	97.3	97.6	99.2	99.0	0.78	0.255	0.074
Crude fat	89.1 ^a	87.6 ^{a,b}	83.7 ^{a,b}	82.0 ^b	1.57	0.019	0.002
Ash	52.4	52.9	50.8	46.7	2.59	0.352	0.116
Phosphorus	59.0	55.5	59.9	49.1	3.26	0.124	0.104
OM ⁵	76.5 ^a	74.1 ^{a,b}	72.4 ^{a,b}	70.4 ^b	1.07	0.007	0.001
CHO ⁶	73.6 ^a	70.9 ^{a,b}	69.3 ^{a,b}	67.4 ^b	1.04	0.004	<0.001

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

Abbreviations: AID, apparent ileal digestibility.

¹Results are given as estimated marginal means ($n = 10$ birds per diet).

²Control diet (control); diets with 10, 20, and 30% crude protein (CP) from *C. jadinii* (CJ10, CJ20, and CJ30, respectively).

³SEM, pooled standard error of the means.

⁴Pregl-Dumas, $N \times 6.25$.

⁵OM: organic matter, calculated as organic matter = dry matter – ash.

⁶CHO: carbohydrate fraction including lignin, calculated as CHO = dry matter – ash – CP – crude fat.

downstream processing methods (Martínez-Force and Benitez, 1995; Nasseri et al., 2011; Øverland and Skrede, 2017). This further implicates that as lignocellulosic hydrolysis and yeast fermentation technology is developed and optimized, the nutritional value of *C. jadinii* in poultry diets may achieve greater potential.

Growth Performance

The effects of yeast and other microbial ingredients, such as bacterial meal, on the growth performance of broiler chickens depend on the yeast or bacterial strain, on the processing conditions, and on the inclusion levels of these ingredients in the diets (Øverland et al., 2010b; Øverland and Skrede, 2017). Similarly, the growth rate of broiler chickens fed with diets supplemented with molasse-produced yeast, constituting 15 to 30% of total

CP, was not affected compared with birds fed with fish meal and SBM-based diets, whereas growth performance was lower in birds fed with diets supplemented with 45% CP from yeast (Daghir and Abdul-Baki, 1977). However, Rodríguez et al. (2011; 2013), reported higher weight gain in broiler chickens fed with diets containing 20 to 22% of CP from distillery vinasse *C. jadinii*, than in birds fed with SBM-based diets. They observed similar weight gain in birds fed with the diets supplemented with 39 to 43% CP from yeast, compared with those fed with SBM-based diets, but weight gain was lower in birds fed with diets supplemented with 59 to 65% yeast CP. On the contrary, broiler chickens fed with diets containing bacterial meal comprising 17 to 34% of dietary CP had higher or similar weight gain than birds fed with SBM-based diets (Schøyen et al., 2007a; Øverland et al., 2010a). In addition, the same strain of *C. jadinii* successfully

Table 6. Effects of increasing dietary levels of *Cyberlindnera jadinii* on the intestinal morphometry of broiler chickens¹.

Morphometry, μm	Diets ²				SEM ³	P-value			
	Control	CJ10	CJ20	CJ30		Overall	Control vs. yeast	Linear	Quadratic
Duodenum									
VH	1,242	1,343	1,268	1,212	76.7	0.656	0.753	0.639	0.313
CD	141	124	109	115	8.8	0.083	0.052	0.024	0.215
VH:CD	9.64	11.45	12.23	11.62	1.08	0.398	0.123	0.179	0.271
Absorption area ⁴	201.8	207.6	186.1	160.1	16.5	0.191	0.356	0.058	0.344
Jejunum									
VH	558	637	593	661	45.6	0.407	0.174	0.202	0.903
CD	88	89	85	96	8.5	0.833	0.797	0.588	0.603
VH:CD	6.63	7.46	7.30	7.34	0.43	0.548	0.081	0.316	0.367
Absorption area ⁴	56.1	63.6	62.2	65.2	6.46	0.783	0.363	0.382	0.736
Ileum									
VH	314 ^a	264 ^b	259 ^b	316 ^a	16.1	0.019	0.111	0.975	0.002
CD	52	50	46	52	2.5	0.334	0.318	0.643	0.127
VH:CD	6.31	5.50	5.88	6.29	0.35	0.316	0.439	0.840	0.089
Absorption area ⁴	19.5	17.7	16.4	21.3	1.27	0.053	0.530	0.472	0.014

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

Abbreviations: CD, crypt depth; VH, villus height.

¹Results are given as least square means of 2 to 5 observations per gut segment per animal, $n = 10$ birds per diet.

²Control diet (control); diets with 10, 20, and 30% crude protein (CP) from *Cyberlindnera jadinii* (CJ10, CJ20, and CJ30, respectively).

³SEM, pooled standard error of the means.

⁴Calculated as $\text{VH} \times \text{villus width}$, expressed as $\mu\text{m}^2 \times 10^{-3}$.

replaced up to 40% of the CP from conventional protein sources in diets for weanling piglets, and the growth performance of the pigs was maintained (Cruz et al., 2019). The different responses to dietary yeast in birds and pigs could be explained by the ability of the pigs to efficiently use nucleic acids in the yeast as components for cell proliferation and restitution of intestinal tissue (Sijben et al., 1998). Yeast contains 3 to 9% nucleic acids (Edozien et al., 1970; Castro et al., 1971; Zee and Simard, 1975), which is considerably higher than the content in SBM and other conventional protein sources (Mateo et al., 2004; Mateo and Stein, 2004). In broiler chickens, purine nucleotides are ultimately degraded to uric acid by the enzyme xanthine oxidase. Uric acid is nonenzymatically metabolized to allantoin in low amounts in birds because it has a negligible uricase enzyme activity (De Boeck and Stockx, 1978; Simoyi et al., 2003). Therefore, the lower BW gain and feed intake of the birds fed with diets supplemented with 30% CP from *C. jadinii* than those of the birds fed with the control diet in our study may be associated with a higher content of nucleic acids such as free adenine derived from *C. jadinii* in the CJ30 diet, which supports the findings of Baker and Molitoris (1974).

The linear decrease in feed intake in the birds fed with diets supplemented with increasing levels of *C. jadinii* during the grower and overall periods in our study could also be explained by the numerically higher pellet durability index in the diets containing *C. jadinii*, as also reported by Abdollahi et al., 2013. The increase in pellet hardness could be due to increased friction and temperature in the pellet press caused by the physical properties of the yeast ingredient, that is, fine particle size, when compared with conventional protein-rich raw materials. In addition, friction forces may have been exacerbated by the reduction in fat content in the yeast-containing diets as a result of balancing the energy content between the dietary treatments. The lower growth performance in birds fed with the CJ20 and CJ30 diets could be a consequence of the high pellet temperatures (>100°C), during feed manufacture, as this may alter the protein structures, promote Maillard reactions and heat damage, and thereby affect the digestibility of CP, AA, and starch and metabolizable energy of diets (Raastad and Skrede, 2003; Pahm et al., 2008; Abdollahi et al., 2010), as well as the availability of lysine, cysteine, and arginine (Ljøkjel et al., 2000). Furthermore, the nutritional evaluation of diets with *C. jadinii* in this study was likely confounded by the increasing pellet temperatures and pellet hardness, which could interfere with nutrient availability and utilization. All things considered, these facts could explain the reduced feed intake and digestibility of nutrients resulting in lower growth performance in the broiler chickens, and therefore, further studies with, for example, similar pellet hardness among dietary treatments are desirable. Alternatively, immunostimulation by dietary yeast may reduce the energy available for growth by repartitioning energy from growth to inflammation and immune pathways, finally leading to reduced growth performance (Fox et al., 2005; Grammes et al., 2013).

Similar to our study, feed intake of broiler chickens decreased with increasing levels of bacterial meal in diets (Schøyen et al., 2007b; Øverland et al., 2010a), which was also reported for broiler chickens fed with diets supplemented with low levels (<2%) of vinasse-grown *C. jadinii* at the expense of SBM (Chand and Ullah Khan, 2014). Both yeast and bacterial meal are rich in nucleic acids when compared with conventional feed ingredients (Castro et al., 1971; Mateo et al., 2004; Mateo and Stein, 2004; Hellwing et al., 2007), which may increase dietary nucleic acid content and reduce feed intake in birds (Kubota and Karasawa, 1994). It was previously shown that feed intake may be depressed in broiler chickens when free adenine comprises 1% of the diet (Baker and Molitoris, 1974). In addition, chitin, constituting 1 to 2% of the yeast cell wall (Kwiatkowski and Edgar, 2012), may reduce feed intake by increasing gastric viscosity, delay gastric emptying, and increase satiety in broiler chickens (Razdan and Pettersson, 1994). However, the measurement of the nucleic acid content and chitin in the yeast or the diets and their effects on feed intake were not the object of this study; thus, further investigation is necessary.

Interestingly, others have reported increased feed intake in broiler chickens fed with diets supplemented with 10 to 30% vinasse-grown *C. jadinii* compared with birds fed with control diets (Rodríguez et al., 2013). High feed intake was also reported for birds fed with low levels of yeast (Shareef and Al-Dabbagh, 2009). Furthermore, feed intake was higher in one- to three-week-old broiler chickens fed with diets supplemented with 17% CP from bacterial meal than in those fed with control diets (Schøyen et al., 2007a). In the latter studies, yeast may have enhanced the palatability of the diets by stimulating umami taste receptors in taste buds (Liu et al., 2018), in a similar way as in fish diets (Kasumyan and Døving 2003; Sahlmann et al., 2019). However, this was not observed in our study, and to our knowledge, no studies relating feed intake to the palatability of yeast in broiler chicken diets are available; thus, further investigation is encouraged.

In similarity to our study, others have shown that FCR increases with increasing levels of yeast protein in diets for broiler chickens. The FCR of broiler chickens fed with diets supplemented with 20.1% CP from dried sugarcane yeast, replacing SBM, was higher than that of birds fed with control diets (Alves Longo et al., 2005). Accordingly, FCR was also higher in broiler chickens fed with diets supplemented with 39 to 65% CP from vinasse-grown *C. jadinii* at the expense of soybean, than in birds fed with the control diet (Rodríguez et al., 2011; 2013). The higher FCR and lower growth performance in birds fed with the yeast-containing diets in our experiment may be explained by an adverse effect of yeast in the utilization of energy in broiler chicken diets (Rodríguez et al., 2011). Interestingly, FCR improved in birds fed with diets supplemented with 17% CP from bacterial protein meal compared with birds fed with SBM-based diets (Schøyen et al., 2007a). As a feed additive, yeast has also shown to

benefit FCR in broiler chickens. Adding lower levels of *S. cerevisiae* yeast (3.5–10.5 g/kg) in SBM-based diets for broiler chickens (Chand and Ullah Khan, 2014) resulted in higher weight gain and lower FCR in the birds fed with increasing amounts of yeast.

Nutritional Evaluation of Diets With *C. jadinii*

The AA composition of *C. jadinii* is similar to that of SBM, but lower in methionine and arginine content than that in SBM; thus, these differences were compensated by adding synthetic AAs to the diets. Inaccurate estimation of the AA availability in *C. jadinii* could be a limitation of the present study as the standardized ileal digestibility values for this ingredient in broiler chickens were not known. This could have led to a potential overestimation of the AA availability and thus reduced weight gain. Yeast cell walls consist of complex polysaccharide structures, resistant to digestion in several species (Roelofsen and Hoette, 1951; Kihlberg, 1972; Wogan, 1975), which may reduce the availability of protein in yeast (Rumsey et al., 1991). Cell walls may contain chitin, which can reduce nutrient absorption, digestibility of fat, and digestibility of CP in broiler chickens (Schiavone et al., 2017). Although no effect of *C. jadinii* on the AID of CP and starch was found, the AID of crude fat was lower in diets with increasing levels of *C. jadinii* than in the control diets. This might be due to an increase in chitin content in the diets derived from *C. jadinii*. Chitin present in yeast cell walls (Kwiatkowski and Edgar, 2012) is a known antinutritional factor that can reduce lipase activity and fat absorption in the small intestine (Razdan and Pettersson, 1994; Kobayashi et al., 2002) and has been associated with reduced digestibility of dry matter in broiler chickens (Khempaka et al., 2006). The lower growth performance in birds fed with the *C. jadinii*-containing diets could also be a result of a reduced proportion of available energy derived from fat. Alternatively, the antioxidant and mycotoxin-binding activity of yeast (Kogan and Kocher, 2007) can improve digestive function by releasing available nutrients bound to antinutritional factors. The lower AID of the carbohydrate fraction in the CJ30 diet might explain the lower growth performance of birds in this group than in those fed with the control diet because carbohydrates are the main energy source for growth. The mineral content of *C. jadinii* varies from 4 to 14%, making yeast a rich source of minerals depending on the used growth media (Rodríguez et al., 2011). The digestibility of ash in the diets in this study was not affected, albeit previous reports of an increase of mineral digestibility in yeast-containing piglet diets (Cruz et al., 2019).

Gut Morphometric Indices and General Health

Intestinal morphology may be positively affected by adding yeast to the diets, although this was not the case in the present study. Previously, the inclusion of

40% CP from *C. jadinii* in diets for young pigs resulted in increased intestinal VH and VH-to-CD ratios (Cruz et al., 2019), which have been associated with improved gut health and increased capacity to digest and absorb nutrients (Laudadio et al., 2012). Mannooligosaccharides in yeast cell walls bind to pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli* and promote their flushing from the gastrointestinal tract, which can balance gut microbiota, prevent infections and toxin formation, and increase nutrient availability and absorption (Ewing and Cole, 1994). The β -glucans in yeast inhibit *E. coli* growth by altering membrane permeability (Rahar et al., 2011), therefore contributing to maintaining normal intestinal function. The high content of β -glucans in the yeast cell wall (50–60%) enhances the functional status of macrophages and neutrophils (Kogan and Kocher, 2007), which can help cope with infections in young birds. On the other hand, increased VH may be a consequence of a greater need for digestive capacity (Svihus, 2014) caused by limited amounts of available nutrients in the gastrointestinal tract. The findings of peritonitis, ascites, and footpad inflammation and general disease in birds fed with diets containing *C. jadinii* (especially replacing 30% of CP) compared with birds in the control group may indicate impaired health status caused by an accumulation of nucleic acids in the birds fed with those diets, although these results did not reach statistical significance and would require further investigation. Alternatively, the cases of footpad inflammation in the CJ30 group may be associated with the slightly higher litter humidity in that group (NS), which predisposes to bacterial proliferation and footpad dermatitis (Eichner et al., 2007).

CONCLUSIONS

C. jadinii grown on local lignocellulosic sugars successfully replaced up to 10% CP from SBM in diets for broiler chickens without compromising growth performance, nutrient digestibility, and intestinal absorptive capacity, showing the potential of *C. jadinii* as a local-based protein source in broiler chicken diets. Replacing SBM with levels higher than 10% of *C. jadinii* on CP basis (20 and 30%) however seemed to reduce the growth performance of broiler chickens. Further research is necessary to explain the mechanisms of action of *C. jadinii* on the digestion and metabolism of broiler chickens and to determine the optimal levels of inclusion of *C. jadinii* in broiler chicken diets.

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